CLAIMS

What is claimed is:

1. A method of screening a candidate compound for susceptibility to biliary excretion, the method comprising the steps of:

(a) providing a culture of hepatocytes, the culture of hepatocytes comprising at least one bile canaliculus;

(b) exposing a candidate compound to the culture; and

(c) determining an amount of candidate compound in the at least one bile canaliculus, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

2. The method of claim 1, wherein the hepatocytes are isolated from a source selected from the group consisting of rat, human, monkey, ape, cat, dog, pig, hog, cattle, oxen, sheep, horses, turkeys, chickens, ducks and geese.

3. The method of claim 1, wherein the culture of hepatocytes further comprises a long-term culture of hepatocytes.

4. The method of claim 1, wherein the culture of hepatocytes further comprises a canalicular network.

5. The method of claim 1, wherein the culture of hepatocytes is further characterized as having a configuration selected from the group consisting of clusters of hepatocytes, aggregates of hepatocytes, at least one layer of hepatocytes, and combinations thereof.

6. The method of claim 5, wherein the hepatocytes are embedded in a matrix.

7. The method of claim 5, wherein the culture of hepatocytes further comprises a sandwich culture of hepatocytes, the sandwich culture comprising at least one layer of hepatocytes and at least one bile canaliculus with the at least one layer of hepatocytes.

8. The method of claim 7, wherein the sandwich culture of hepatocytes further comprises a long-term sandwich culture of hepatocytes.

9. The method of claim 7, wherein the at least one layer of hepatocytes is sandwiched between two layers of matrix.

10. The method of claim 9, wherein the matrix is selected from the group consisting of a biological matrix medium, a synthetic matrix medium, and combinations thereof.

11. The method of claim 10, wherein the biological matrix medium is selected from the group consisting of collagens, laminins, basement membrane-derived complexes, derivatives thereof and combinations thereof.

12. The method of claim 1, wherein the amount of the candidate compound in the at least one bile canaliculus is determined by calculating a biliary clearance value for the culture, the calculated biliary clearance value indicating the susceptibility of the candidate compound to biliary excretion.

13. A method of screening a plurality of candidate compounds simultaneously for susceptibility to biliary excretion, the method comprising:

(a) providing a plurality of cultures of hepatocytes, wherein each culture of hepatocytes comprises at least one bile canaliculus;

(b) exposing a different candidate compound within the plurality of candidate compounds to each culture within the plurality of cultures; and

(c) determining an amount of candidate compound in the at least one bile canaliculus within each culture, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

14. The method of claim 13, wherein the hepatocytes are isolated from a source selected from the group consisting of rat, human, monkey, ape, cat, dog, pig, hog, cattle, oxen, sheep, horses, turkeys, chickens, ducks and geese.

15. The method of claim 13, wherein the cultures of hepatocytes further comprise long-term cultures of hepatocytes.

16. The method of claim 13, wherein the cultures of hepatocytes each further comprise a canalicular network.

17. The method of claim 13, wherein the cultures of hepatocytes are further characterized as having a configuration selected from the group consisting of clusters of hepatocytes, aggregates of hepatocytes, at least one layer of hepatocytes, and combinations thereof.

18. The method of claim 17, wherein the hepatocytes are embedded in a matrix.

19. The method of claim 17, wherein each culture of hepatocytes further comprises a sandwich culture of hepatocytes, the sandwich culture

comprising at least one layer of hepatocytes and at least one bile canaliculus with the at least one layer of hepatocytes.

20. The method of claim 19, wherein each sandwich culture of hepatocytes further comprises a long-term sandwich culture of hepatocytes.

21. The method of claim 19, wherein the at least one layer of hepatocytes is sandwiched between two layers of matrix.

22. The method of claim 21, wherein the matrix is selected from the group consisting of a biological matrix medium, a synthetic matrix medium, and combinations thereof.

23. The method of claim 22, wherein the biological matrix medium is selected from the group consisting of collagens, laminins, basement membrane-derived complexes, derivatives thereof and combinations thereof.

24. The method of claim 13, wherein the amount of the candidate compound in the at least one bile canaliculus is determined by calculating a biliary clearance value for the culture, the calculated biliary clearance value indicating the susceptibility of the candidate compound to biliary excretion.

25. A method of screening a candidate compound for susceptibility to biliary excretion, the method comprising the steps of:

(a) providing a culture of hepatocytes, the culture comprising at least one bile canaliculus;

(b) exposing a candidate compound and a pre-selected amount of a marker compound to the culture for a time sufficient to allow uptake;

(c) washing the culture; and

(d) detecting an amount of marker compound present in the at least one bile canaliculus in the culture, the presence or the absence of a reduced amount of the marker compound as compared to the pre-selected amount of marker compound indicating the susceptibility of the candidate compound to biliary excretion.

26. The method of claim 25, wherein the hepatocytes are isolated from a source selected from the group consisting of rat, human, monkey, ape, cat, dog, pig, hog, cattle, oxen, sheep, horses, turkeys, chickens, ducks and geese.

27. The method of claim 25, wherein the culture of hepatocytes further comprises a long-term culture of hepatocytes.

28. The method of claim 25, wherein the culture of hepatocytes further comprises a canalicular network.

29. The method of claim 25, wherein the culture of hepatocytes is further characterized as having a configuration selected from the group consisting of clusters of hepatocytes, aggregates of hepatocytes, at least one layer of hepatocytes, and combinations thereof.

30. The method of claim 29, wherein the hepatocytes are embedded in a matrix.

31. The method of claim 29, wherein the culture of hepatocytes further comprises a sandwich culture of hepatocytes, the sandwich culture comprising at least one layer of hepatocytes and at least one bile canaliculus with the at least one layer of hepatocytes.

32. The method of claim 31, wherein the sandwich culture of hepatocytes further comprises a long-term sandwich culture of hepatocytes.

33. The method of claim 32, wherein the at least one layer of hepatocytes is sandwiched between two layers of matrix.

34. The method of claim 33, wherein the matrix is selected from the group consisting of a biological matrix medium, a synthetic matrix medium, and combinations thereof.

35. The method of claim 34, wherein the biological matrix medium is selected from the group consisting of collagens, laminins, basement membrane-derived complexes, derivatives thereof and combinations thereof.

36. The method of claim 26, wherein the marker compound is selected from the group consisting of a fluorogenic compound, a fluorescent compound, a chemiluminescent compound, a colorimetric compound, a radiolabeled compound and combinations thereof.

37. The method of claim 26, wherein steps (a) through (d) are carried out in at least one well of a multi-well plate.

38. The method of claim 26, further comprising screening a plurality of candidate compounds simultaneously for susceptibility to biliary excretion.

39. A method of screening a candidate compound for susceptibility to biliary excretion, the method comprising the steps of:

(a) establishing first and second cultures of hepatocytes, each culture comprising at least one bile canaliculus, the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi;

(b) exposing a candidate compound to the first culture and to the second culture for a time sufficient to allow uptake of the candidate compound;

(c) washing and then lysing the first and second cultures;

(d) measuring an amount of candidate compound present in a lysate obtained from each culture in step (c); and

(e) calculating a biliary clearance value derived from the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi using the amount of candidate compound in each culture lysate measured in step (d), the calculated biliary clearance value indicating the susceptibility of the candidate compound to biliary excretion.

40. The method of claim 39, wherein the hepatocytes are isolated from a source selected from the group consisting of rat, human, monkey, ape, cat, dog, pig, hog, cattle, oxen, sheep, horses, turkeys, chickens, ducks and geese.

41. The method of claim 39, wherein the first and second cultures of hepatocytes each further comprise a long-term culture of hepatocytes.

42. The method of claim 39, wherein the first and second cultures of hepatocytes further comprise a canalicular network.

43. The method of claim 39, wherein the first and second cultures of hepatocytes are further characterized as having a configuration selected from the group consisting of clusters of hepatocytes, aggregates of hepatocytes, at least one layer of hepatocytes, and combinations thereof.

44. The method of claim 39, wherein the hepatocytes are embedded in a matrix.

45. The method of claim 39, wherein the first and second cultures of hepatocytes further comprise a sandwich culture of hepatocytes, the sandwich culture comprising at least one layer of hepatocytes and at least one bile canaliculus with the at least one layer of hepatocytes.

46. The method of claim 45, wherein the at least one layer of hepatocytes is sandwiched between two layers of matrix.

47. The method of claim 45, wherein the first and second cultures of hepatocytes each further comprise a long-term culture of hepatocytes.

48. The method of claim 46, wherein the matrix is selected from the group consisting of a biological matrix medium, a synthetic matrix medium, and combinations thereof.

49. The method of claim 48, wherein the biological matrix medium is selected from the group consisting of collagens, laminins, basement membrane-derived complexes, derivatives thereof and combinations thereof.

50. The method of claim 39, wherein steps (a) through (d) are carried out in at least one well of a multi-well plate.

51. The method of claim 39, further comprising screening a plurality of candidate compounds simultaneously for susceptibility to biliary excretion.

52. A method of screening a metabolite of a candidate parent compound for susceptibility to biliary excretion, the method comprising the steps of:

(a) establishing a first set and second set of two cultures of hepatocytes, each culture comprising at least one bile canaliculus, a first culture within each set having intact bile canaliculi and a second culture within each set having disrupted bile canaliculi;

(b) exposing a candidate parent compound to the first culture and to the second culture of each set for a time sufficient to allow uptake of the candidate compound;

(c) inducing metabolic enzyme activity in the hepatocytes of the first set of cultures;

(d) washing and lysing the first and second cultures of each set;

(e) measuring an amount of candidate parent compound present in a lysate obtained from each culture in step (d);

(f) measuring an amount of the metabolite of the candidate parent compound present in a lysate obtained from each culture in step (d);

(g) calculating a biliary clearance value derived from the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi using the amount of candidate parent compound in each culture lysate measured in step (e), the calculated biliary clearance value indicating the susceptibility of the candidate parent compound to biliary excretion; and

(h) calculating a biliary clearance value derived from the first culture having intact bile canaliculi and the second culture having

disrupted bile canaliculi using the amount of the metabolite of the candidate parent compound in each culture lysate measured in step (f), the calculated biliary clearance value indicating the susceptibility of the metabolite of the candidate parent compound to biliary excretion.

53. The method of claim 52, wherein the hepatocytes are isolated from a source selected from the group consisting of rat, human, monkey, ape, cat, dog, pig, hog, cattle, oxen, sheep, horses, turkeys, chickens, ducks and geese.

54. The method of claim 52, wherein the first and second sets of cultures of hepatocytes each further comprise a long-term culture of hepatocytes.

55. The method of claim 52, wherein the first and second sets of cultures of hepatocytes further comprise a canalicular network.

56. The method of claim 52, wherein the cultures of hepatocytes are further characterized as having a configuration selected from the group consisting of clusters of hepatocytes, aggregates of hepatocytes, at least one layer of hepatocytes, and combinations thereof.

57. The method of claim 56, wherein the hepatocytes are embedded in a matrix.

58. The method of claim 56, wherein each culture of hepatocytes further comprises a sandwich culture of hepatocytes, the sandwich culture comprising at least one layer of hepatocytes and at least one bile canaliculus with the at least one layer of hepatocytes.

59. The method of claim 58, wherein the at least one layer of hepatocytes is sandwiched between two layers of matrix.

60. The method of claim 58, wherein the cultures of hepatocytes each further comprise a long-term culture of hepatocytes.

61. The method of claim 59, wherein the matrix is selected from the group consisting of a biological matrix medium, a synthetic matrix medium, and combinations thereof.

62. The method of claim 61, wherein the biological matrix medium is selected from the group consisting of collagens, laminins, basement membrane-derived complexes, derivatives thereof and combinations thereof.

63. The method of claim 52, wherein steps (a) through (f) are carried out in at least one well of a multi-well plate.

64. The method of claim 52, further comprising screening a plurality of candidate parent compounds and a plurality of metabolites of the candidate parent compounds simultaneously for susceptibility to biliary excretion.